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Conidiobolus antarcticus, a synonym of *C. osmodes*

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Abstract—DNA from two ribosomal regions (nrLSU and mtSSU) and one protein coding region (RPB2) was sequenced and analysed for ten *Conidiobolus* isolates, including the ex-type isolates of *C. antarcticus* and *C. osmodes*, and a detailed morphological comparison of these two species was compiled. *Conidiobolus antarcticus* formed a highly supported (100%) clade with three *C. osmodes* isolates in the phylogenetic tree based on the combined data sets of these three genes. The two species were similar in the colony diameter, hyphal diameter, primary conidial size and shape, and zygospore formation, size, and shape. Therefore, we consider *C. antarcticus* to be a synonym of *C. osmodes*.

Key words—*Ancylistaceae, Entomophthorales,* taxonomy

Introduction

Conidiobolus antarcticus, isolated from the Antarctic mosses *Scistidium antarctici* and *Hennediella heimii*, was first described by Tosi & al. (2004). In size and shape of the primary conidia and zygospores, *C. antarcticus* resembles *C. osmodes* (Drechsler 1954), isolated from decaying plant detritus in Louisiana, U.S.A. When we carried out a study on the phylogenetic systematics of *Conidiobolus*, we included the ex-type cultures of *C. antarcticus* and *C. osmodes*. A three-gene molecular analysis and morphological comparisons indicated that these two species are conspecific.

Materials & methods

Fungal isolates and culturing conditions

The isolates were provided by the USDA-ARS Entomopathogenic Fungus Collection, Ithaca, New York, U.S.A. (ARSEF) and the Research Center of Entomopathogenic

Table 1. *Conidiobolus* and *Basidiobolus* species and strains used in this study, with GenBank accession numbers.

Species	Strain	nrLSU	RPB2	mtSSU
C. antarcticus	ARSEF 6913 ^{T}	DQ364207	DQ364217	DQ364227
C. bangalorensis	ARSEF 449 ^{T}	DQ364204	DQ364214	DQ364225
C. coronatus	ARSEF 206	DQ364201	DQ364211	DQ364221
	ARSEF 421	DQ364203	DQ364213	DQ364223
	ARSEF 525	DQ364205	DQ364215	DQ364224
C. lamprauges	ARSEF 2338	DQ364206	DQ364216	DQ36422
C. osmodes	RCEF 4447	JN131539	MH401096	MH401095
	ARSEF 79	DQ364199	DQ364209	DQ364219
	ARSEF 193 ^{T}	DQ364200	DQ364210	DQ364220
C. thromboides	ARSEF 70	DQ364198	DQ364208	DQ364218
B. microsporus	ARSEF 265	DQ364202	DQ364212	DQ364222

^{T}, ex-type culture

Fungus, Hefei, Anhui, China (RCEF). The species, strains, and GenBank sequence accession numbers are given in Table 1.

The mycelium was transferred to a Petri dish containing a Sabouraud dextrose agar yeast extract culture medium (SDAY; 4% dextrose, 1% peptone, 1% yeast extract, 1.5% agar) and covered with autoclaved cellophane. The Petri dish was sealed with parafilm and placed in an incubator at 25°C for approximately 1–2 weeks or until the fungus had grown to the edge of the cellophane. The mycelia were scraped from the cellophane and stored at –20°C until DNA extraction.

DNA extraction, amplification, and sequencing

Mycelia were ground in a sterile 1.5 mL microcentrifuge tube with a small plastic stick. The extraction was performed using benzyl chloride according to the method of Zhu & al. (1994). All DNA samples were then diluted 1:20 for PCR reactions and stored at –10°C.

Regions of three genes were amplified by PCR: nuclear ribosomal large subunit (nrLSU) by primers LR0R and LR5 (Vilgalys & Hester 1990), mitochondrial small subunit of rDNA (mtSSU) by MtSSUc-2F and MtSSUc-2R (http://aftol.biology.duke.edu/pub/primers/getPrimerDetails?primer_id=258), and RNA polymerase unit II second large subunit (RPB2) by fRPB2-7F and RPB2-11aR (Liu & al. 1999). All procedures used in this study for LSU amplification have been described previously (Liu & al. 2005).

The PCR reaction mixture for mtSSU contained 200 µM each dNTP, 1X Mg-free buffer, 2.5 mM $MgCl_2$, 0.5 µM each primer, 3% DMSO, 10–50 ng genomic DNA and 0.04 Unit/ µL Taq polymerase (Promega). The PCR program comprised an initial denaturation step of 95°C for 5 min, 34 cycles of 94°C for 1 min, 54°C for 2 min, 72°C for 2 min, and a final extension at 72°C for 10 min.

The PCR reaction mixture for RPB2 was the same as the mtSSU mixture, but without DMSO. A hot start PCR technique was performed, and the PCR conditions were 100°C for 5 min, an initial denaturation step 95°C for 5 min (during which Taq polymerase was added to each tube), 34 cycles of 94°C for 1 min, 49.2°C for 2 min, 72°C for 2 min, and a final extension at 72°C for 10 min.

PCR products were purified using the QIAquick PCR purification kit® and sequenced in both directions using the initial amplification primers at the Biotechnology Resource Center at Cornell University.

Phylogenetic analysis

The sequences were aligned using Clustal 1.83 (Thompson & al. 1994), and the alignment was refined by eye. *Basidiobolus microsporus* R.K. Benj. was used as the outgroup species. Parsimony analysis was performed in PAUP* version b10 (Swofford 2002) using a heuristic search. A starting tree was obtained via a 'closest' addition sequence, tree-bisection-reconnection was used as the branch-swapping algorithm, and MULTREES was switched off. Character states were unordered, and characters were equally weighted. Bootstrap values were calculated based on 1000 replicates of a heuristic fast stepwise addition search.

Bayesian analysis was carried out using MrBayes 3.0b4 (Huelsenbeck 2000, Huelsenbeck & al. 2001). We used a six-parameter model to run four chains for 500,000 generations, saving a tree every 100 generations. The first 500 trees were discarded (burn in), and the remaining trees were saved to a file. A 50% majority rule consensus tree was then calculated using PAUP*. Tree topology matches the combined parsimony analysis.

Results & discussion

nrLSU phylogeny

Plate 1a

Parsimony analysis of the nrLSU data set yielded one Maximum Parsimony tree (MPT) of 865 steps with CI = 0.884 and RI = 0.902. *Conidiobolus antarcticus* formed an unresolved clade with the three *C. osmodes* isolates in the strict consensus. There was 99% identity between *C. antarcticus* and *C. osmodes* ARSEF79 (923/925 due to two point indels, both in *C. antarcticus*) according to a Pairwise Blast. Among the three isolates of *C. osmodes*, the gene sequences differed by 0–1 base positions.

mtSSU phylogeny

Plate 1b

Parsimony analysis of the mtSSU data set yielded one MPT of 177 steps with CI = 0.927 and RI = 0.940. *Conidiobolus antarcticus* formed a clade with the three *C. osmodes* isolates. There was 100% identity among the isolates of *C. osmodes* and *C. antarcticus*.

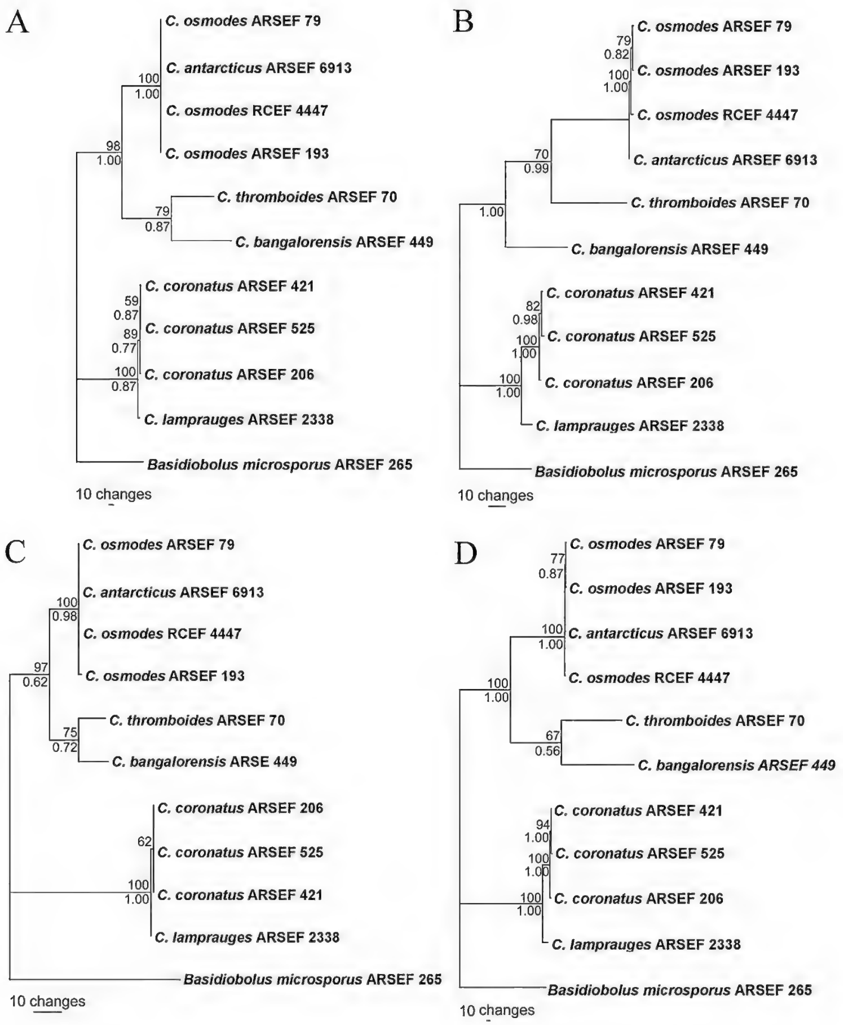

Plate 1. Phylogenetic relationships among ten strains from six *Conidiobolus* species, based on the data set from different genes, with *Basidiobolus microsporus* as outgroup. Bootstrap proportions (BP) and Bayesian posterior probabilities (PP) >50% are indicated above the branches. A. Tree based on the nrLSU gene. B. Tree based on the mtSSU gene. C. Tree based on the RPB2 gene. D. Tree based on the combined data set of these three genes.

RPB2 phylogeny

PLATE 1C

Parsimony analysis of the RPB2 data set yielded one MPT of 621 steps with CI = 0.773 and RI = 0.820. *Conidiobolus antarcticus* formed an unresolved clade with the three *C. osmodes* isolates in the strict consensus. The relative positions of *C. bangalorensis* and *C. thromboides* differed from the other two gene trees, but the trees were not in significant conflict. There was 99% identity between *C. antarcticus* and *C. osmodes* ARSEF79 (869/875 due to mismatches: C:N, T:C, A:C, T:N, T:C, –:A) according to a Pairwise Blast. Among the three isolates of *C. osmodes*, the gene sequences differed by 0–6 base positions, and the three *C. osmodes* isolates sequences were 99% identical.

Combined LSU, mtSSU and RPB2 phylogeny

PLATE 1D

One MPT of 1647 steps with CI = 0.856 and RI = 0.882 was obtained from the parsimony analysis of the combined data set. *Conidiobolus antarcticus* formed a short clade with the three *C. osmodes* isolates with 100% bootstrap support. Bayesian and parsimony analyses both gave no resolution within the *C. osmodes* and *C. antarcticus* clades.

Conidiobolus antarcticus was separated from *C. osmodes* type isolates by a few substitutions and indels, but these interspecies differences in nucleotide sequence fell within the range of infrageneric nucleotide divergence among the three *C. osmodes* isolates. The three *C. coronatus* isolates showed similarly wide infraspecific differences: LSU sequences differed by 0–2 base positions; mtSSU sequences did not differ; and PRB2 sequences differed by 5–10 base positions.

The analyses of phylogenetic tree and nucleotide divergence support *Conidiobolus antarcticus* and *C. osmodes* as the same species at the DNA level.

Morphological analysis

Tosi & al. (2004) reported that the morphological features of *Conidiobolus antarcticus* to some extent resembled those of *C. megalotocus* Drechsler, *C. thromboides* Drechsler, *C. bangalorensis* Sriniv. & Thirum., and *C. lamprauges* Drechsler, but our analysis of sequences from three gene regions reveals that *C. antarcticus* is clearly not closely related to *C. thromboides*, *C. bangalorensis*, or *C. lamprauges*. Instead, the phylogenetic tree showed a very close relationship between *C. antarcticus* and *C. osmodes*.

TABLE 2 provides a comparative summary of the morphology of *C. antarcticus* (from Tosi & al. 2004) and *C. osmodes* (from Drechsler 1954). These two species are morphologically similar in colony diameter, hyphal diameter, primary conidial size and shape, and zygospore formation, size, and shape of the zygospore; there are only slight differences in vegetative

Table 2. Morphological comparison of *Conidiobolus antarcticus* and *C. osmodes* (data from Tosi et al. 2004 and Drechsler 1954)

Character	*C. antarcticus*	*C. osmodes*
Colony diameter	c. 42 mm in 5 days	c. 30 mm in 4 days
Hyphal diameter	8–(10)–12 µm	4–12 µm
Hyphal segments	Continuous, but disjointed putting forth (3–4) short diverticulate brancheS	Becoming septate and noncontinuous
Primary conidia	Pyriform to globose, 25–31 × 20–25 µm, with 5 µm papilla	Globose to obovoid, 25–37 × 22–30 µm, with 2–5 µm papilla
Zygospores	Globose, 25–40 µm, smooth, thick-double walled (2.5–5 µm)	Globose to ellipsoidial, 13–37 µm, sometimes smooth but usually bearing smallish ridges, thick-double walled (2–6.5 µm)
Zygospore formation	Conjugation between two segments of separate hyphae or same hypha	Between conjugating cell of different or same hyphae or hyphal bodies
Odor	Not reported	Benzene hexachloride

appearance and colony odor. *Conidiobolus osmodes* RECF 4447 was checked for key morphological features, including colonial diameter (c. 33 mm in 4 d), primary conidia (25–38 × 22–33 µm), and zygospores (25–30 µm with thick-double wall 3–5 µm). These features resembled those of *C. antarcticus.*

We consider that the relevant morphological characteristics and sequences from three genes provide sufficient evidence to place *C. antarcticus* and *C. osmodes* in synonymy. Consequently, our taxonomic proposal is:

Conidiobolus osmodes Drechsler, Amer. J. Bot. 41: 571. 1954.

= *Conidiobolus antarcticus* S. Tosi, Caretta & Humber, Mycotaxon 90(2): 344. 2004.

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